

In the Specification:

At page 1, delete the third paragraph (lines 12-14).

At page 1, line 5, before "Statement" insert the following paragraph:

Reference to Related Applications.

$\epsilon_1$  This application is a continuation-in-part of PCT/US98/10080, filed May 15, 1998, which is a continuation-in-part of U.S.S.N. 08/888,534, filed July 7, 1997, and U.S.S.N. 08/857,076, filed May 15, 1997, issued as U.S. Patent No. 6,225,120 on May 1, 2001.

At page 83, replace the second paragraph (lines 14-26) with the following replacement paragraph:

Score = 151 (68.4 bits), Expect = 1.9e-140, Sum P(8) = 1.9e-140  
Identities = 28/54 (51%), Positives = 38/54 (70%)

$\epsilon_2$  SEQ ID NO: 161 AFX:226 SPVGHFAKWSGSPCSRNREEADMWTTFRPRSSSNASSVSTRLSPLRPESEVLAE279  
SEQ ID NO: 162 SP F+KW SP S + ++ D W+TFRPR+SSNAS++S RLSP+ E + L E  
SEQ ID NO: 163 FKHR:287 SPGSQFSKWPASPGSHSNDDFDNWSTFRPRTSSNASTISGRSLSPIMTEQDDLGE340  
SEQ ID NO: 164 DAF-16a SFRPRTQSNLSIPGSSS

Score = 132 (59.8 bits), Expect = 1.9e-140, Sum P(8) = 1.9e-140  
Identities = 22/42 (52%), Positives = 28/42 (66%)

SEQ ID NO: 165: AFX: 7 KAAAIIDLDPDFEQSRPRSCTWPLPRPEIANQPSEPPEVEP 48  
SEQ ID NO: 166 +A++++DPDFEP RPRSCTWPLPRPE + S P  
SEQ ID NO: 167 FKHR: 3 EAPQVVEIDPDFEPLPRPRSCTWPLPRPEFSQSN SATSSPAP 44  
SEQ ID NO: 168 DAF-16 TFMNTDDVMMNDMEPIPRDRCNTWPMRRPQLEPPLNSSP 177  
SEQ ID NO: 169 T ++P+ V ++ D EP+PR R TWP+ RP++ + +++++

Beginning at page 97, replace page 97, second paragraph (lines 3-37), page 98, page 99, and page 100, first paragraph (lines 1-4) with the following replacement paragraphs:

Score = 252 (88.7 bits), Expect = 2.2e-60, Sum P(6) = 2.2e-60  
Identities = 47/80 (58%), Positives = 60/80 (75%), Frame = +3

$\epsilon_3$  SEQ ID NO:170 Query:439 LEQAGGNPWHQFVENNLILKMGFVDRKRGFLFARRRQLLLTEGPHLYYVDPVNVKVLKGEI 498  
SEQ ID NO:171 LE+Q NP+H F N+LILK G ++K++GLFARRR LLTEGPHL Y+D N VLKGE+  
SEQ ID NO:172 Sbjct:1818 LEEQRVKNPFFHIFTNNSLILKQGYLEKKRGLFARRRMFLLLTEGPHLLYIDVPNLVLKGEV1997

SEQ ID NO:170 Query: 499 PWSQELRPEAKNFKTFFVHT 518  
SEQ ID NO:171 PW+ ++ E KN TFF+HT  
SEQ ID NO:172 Sbjct: 1998 PWTPCMQVELKNSGTFFIHT 2057

Score = 201 (70.8 bits), Expect = 2.2e-60, Sum P(6) = 2.2e-60  
Identities = 48/123 (39%), Positives = 72/123 (58%), Frame = +1

SEQ ID NO:173 Query: 263 SDLWALGCIIYQLVAGLPPFRAGNEYLIFQKIIKLEYDFPEKFFPKARDLVEKLLVLDAT 322  
SEQ ID NO:174 +D+W LGCI++Q +AG PPFR N+Y + ++I +L++ FPE F +A +++ K+LV  
SEQ ID NO:175 Sbjct: 802 TDIWGLGCILFQCLAGQPPFRAVNQYHLLKRIQELDFSFPFEGFPEEASEIIAKILV--G\*H 978

SEQ ID NO:173 Query: 323 KRLGCE----EMEGYGP-----LKAHPFFESVTWENLHQQTTPPKLTAYLPAMSEDDE 370  
SEQ ID NO:174 + L E ++ P L AH FFE+V W N+ PP L AY+PA + E  
SEQ ID NO:175 Sbjct: 979 ETLKTEYVIFNLQVRDPSTRITSQELMAHKFFENVDWVNIANIKPPVLHAYIPATFGEPE1158

SEQ ID NO:173 Query: 371 DCYGN 375  
SEQ ID NO:174 Y N  
SEQ ID NO:175 Sbjct: 1159 -YYSN 1170

Score = 180 (63.4 bits), Expect = 2.2e-60, Sum P(6) = 2.2e-60  
Identities = 31/72 (43%), Positives = 52/72 (72%), Frame = +2

SEQ ID NO:176 Query: 157 FGLSYAKNGELLKYIRKIGSFDECTCTRFYTAEIVSALEYLHGKGIHRDLKPENILLNED 216  
SEQ ID NO:177 F + +NG+L + + GSFD ++F+ +EI++ L++LH I+HRD+KP+N+L+ +D  
SEQ ID NO:178 Sbjct: 287 FVIGLVENGDLGESLCHFGSFDMLTSKFFASEILTGLQFLHDNKIVHRDMKPDNVLIQKD 466

SEQ ID NO:176 Query: 217 MHIQITDFGTAK 228  
SEQ ID NO:177 HI ITDFG+A+  
SEQ ID NO:178Sbjct: 467 GHILITDFGSAQ 502

Score = 83 (29.2 bits), Expect = 2.2e-60, Sum P(6) = 2.2e-60  
Identities = 15/53 (28%), Positives = 32/53 (60%), Frame = +

SEQ ID NO:179 Query:108 YAIKILEKRHHIENKVPYVTRERDVMSRLD----HPFFVKLYFTFQDDEKL 155  
SEQ ID NO:180 +A+K+L+K ++ + K+ + RE++++ L HPF +LY F D ++  
SEQ ID NO:181 Sbjct: 8 FAVKVLQKSYLNRHQMDAIREKNILTYLSQECGHPFVTQLYTHFDQARI 166

Score = 81 (28.5 bits), Expect = 2.2e-60, Sum P(6) = 2.2e-60  
Identities = 15/29 (51%), Positives = 19/29 (65%), Frame = +2

SEQ ID NO:182 Query: 519 PNRYYLMDPSGNAHKWCRKIQEVWRQRY 547  
SEQ ID NO:183 PNR YYL D A +WC+ I +V R+RY  
SEQ ID NO:184 Sbjct: 2129 PNRVYYLFDLEKKADEWCKAINDV-RKRY 2212

Score = 78 (27.5 bits), Expect = 2.2e-60, Sum P(6) = 2.2e-60  
Identities = 15/25 (60%), Positives = 18/25 (72%), Frame = +3

SEQ ID NO:185 Query: 232 PESKQARANSFVGTAQYVSPPELLTE 256  
SEQ ID NO:186 PE AR +FVGTA YVSPE+L +  
SEQ ID NO:187 Sbjct: 660 PEENTARRTTFVGTALYVSPEMLAD 734

Overall, *C. elegans* *pdk-1* exhibits the following homology to human PDK-1.

Score = 118 (54.4 bits), Expect = 1.4e-104, Sum P(5) = 1.4e-104  
Identities = 21/62 (33%), Positives = 41/62 (66%)

SEQ ID NO:188 Query:63 KRTSNDMFLQSMGEGAYSQVFRCREVATDAMFAVKVLQKSYLNRHQMDAIREKNILT 122  
SEQ ID NO:189 K+ DF F + +GEG++S V RE+AT +A+K+L+K ++ + K+ + RE++++  
SEQ ID NO:190 Sbjct:76 KKRPEDFKFGKILGEGSFSTVVLARELATSREYAIKILEKRHHIENKVPYVTRERDVMS 135

SEQ ID NO:188 Query: 123 YL 124  
 SEQ ID NO:189 L  
 SEQ ID NO:190 Sbjct: 136 RL 137

Score = 230 (106.0 bits), Expect = 1.4e-104, Sum P(5) = 1.4e-104  
 Identities = 39/90 (43%), Positives = 63/90 (70%)

SEQ ID NO:191 Query: 131 HPFVTQLYTHFDQARIYFVIGLVENGDLGESLCHFGSFDMLTSKFFASEILTGLQFLHD 190  
 SEQ ID NO:192 HPF +LY F D ++YF + +NG+L + + GSFD ++F+ +EI++ L++LH  
 SEQ ID NO:193 Sbjct: 139 HPFFVKLYFTFQDDEKLYFGLSYAKNGELLYKIRKIGSFDETCTRFYTAEIVSALEYLHG 198

SEQ ID NO:191 Query: NKIVHRDMKPDNVLIQKDGHILITDFGSAQ 220  
 SEQ ID NO:192 I+HRD+KP+N+L+ +D HI ITDFG+A+  
 SEQ ID NO:193 Sbjct: KGIHRDLKPENILLNEDMHIQITDFGTAK 228

Score = 238 (109.7 bits), Expect = 1.4e-104, Sum P(5) = 1.4e-104  
 Identities = 43/98 (43%), Positives = 67/98 (68%)

SEQ ID NO:194 Query: 259 EENTARRTTFVGTALYVSPEMLADGDVGPQTDIWLGCILFQCLAGQPPFRAVNQYHLLK 318  
 SEQ ID NO:195 E AR +FVGTA YVSPE+L + +D+W LGCI++Q +AG PPFRA N+Y + +  
 SEQ ID NO:196 Sbjct: 233 ESKQARANSFVGTAQYVSPPELLTEKSACKSSDLWALGCIYQLVAGLPPFRAGNEYLIFQ 292

SEQ ID NO:194 Query: 319 RIQELDFSPEGFPPEEASEIIAKILVRDPSTRITSQEL 356  
 SEQ ID NO:195 +I +L++ FPE F +A +++ K+LV D + R+ +E+  
 SEQ ID NO:196 Sbjct: 293 KIIKLEYDFPEKFFPKARDLVEKLLVLDATKRLGCEEM 330

Score = 85 (39.2 bits), Expect = 1.4e-104, Sum P(5) = 1.4e-104  
 Identities = 17/35 (48%), Positives = 21/35 (60%)

SEQ ID NO:197 Query: 356 LMAHKFFENVVDVWNIANIKPPVLHAYIPATFGEPE 390  
 SEQ ID NO:198 L AH FFE+V W N+ PP L AY+PA + E  
 SEQ ID NO:199 Sbjct: 336 LKAHPFFESVTWENLHQTPPKLTAYLPAMSEDDE 370

Score = 324 (149.3 bits), Expect = 1.4e-104, Sum P(5) = 1.4e-104  
 Identities = 59/104 (56%), Positives = 75/104 (72%)

SEQ ID NO:200 Query: 458 LEEQRVKNPFFHIFTNNSLILKQGYLEKKRGLFARRRMFLLTEGPHLLYIDVPNLVLKGEV 517  
 SEQ ID NO:201 LE+Q NP+H F N+LILK G ++K++GLFARRR LLTEGPHL Y+D N VLKGE+  
 SEQ ID NO:202 Sbjct: 439 LEKQAGGNPWHQFVENNLILKMGFVDKRGFLFARRRQLLLTEGPHLYYVDPNVKVLKGEI 498

SEQ ID NO:200 Query: 518 PWTPCMQVELKNSGTFIHTPNRVYVYLFDEKKADEWCKAINDV 561  
 SEQ ID NO:201 PW+ ++ E KN TFF+HTPNR YYL D A +WC+ I +V  
 SEQ ID NO:202 Sbjct: 499 PWSQELRPEAKNFKTFFVHTPNRTYYLMDPSGNAHKWCRKIQEV 542

Mapping of the *mg142* mutation to this open reading frame establishes the function of this protein. It is much more closely related to PDK than to any other known kinase. PDK is a mammalian kinase that phosphorylates an essential serine residue on AKT, contributing to its activation. The region of *akt-1* phosphorylated by PDK-1 is shown below (SEQ ID NO: 203-207 and 305).

SEQ ID NO:203 human AKT 276 KLENLMLDKDGHIKITDFGLCKEGIKDGATMKTFCGTPEYLAPEV 320  
 SEQ ID NO:204 KLENL+LDKDGHIKI DFGLCKE I G TFCGTPEYLAPEV  
 SEQ ID NO:205 Ce akt-133509 KLENLLLDKDGHIKIADFGLCKEEISFGDKTSTFCGTPEYLAPEV 33643

E3  
Corl.

SEQ ID NO:206 Ceakt2 326 LCKEEIKYGDKTSTFCGTPEYLAPEVIEDIDYDRSVDWWGVGVVMMCGRLPFSKENGK  
SEQ ID NO:207 LCKE I G TFCGTPEYLAPEV+ED DYR+VDWWG+GVVMMCGRLPF +++ +  
SEQ ID NO:305 moAKT: 298 LCKEGISDGMTKFTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMMCGRLPFYNQDHER

Replace page 178, fourth paragraph, lines 21-24, page 179-186, and page 187, first paragraph, lines 1-14, with the following replacement paragraphs:

Pepck

>R11A5

Length = 26,671

Plus Strand HSPs:

Score = 994 (461.5 bits), Expect = 0.0, Sum P(5) = 0.0

Identities = 176/223 (78%), Positives = 195/223 (87%), Frame = +1

SEQ ID NO:211 Query:201 AKNNGEFVRCVHVSQGPKPVATKVINHWPCNPEKTIIAHRPAEREIWSFGSGYGGNSLLG 260  
SEQ ID NO:212 A N +FVRC+HSV G P+PV +VINHWPCNPE+ +IAHRP EREIWSFGSGYGGNSLLG  
SEQ ID NO:213Sbjct:8682 ALGNQDFVRCIHSVGLPRPVKQRVINHWPCNPERVLIARPPEREIWSFGSGYGGNSLLG 18861

SEQ ID NO:211 Query: 261 KKCFFALRIAMNIGYDEGWMAEHMLIMGVTSFKGEERFVAAAFPSACGKTNLAMLEPTIPG 320  
SEQ ID NO:212 KKCFFALRIA NI DEGWMAEHMLIMGV T P G E F+AAAFPSACGKTNLAMLEPT+PG  
SEQ ID NO:213 Sbjct:18862 KKCFFALRIASNIADDEGWMAEHMLIMGVTRPCGREHFIAAFPSACGKTNLAMLEPTLPG 19041

SEQ ID NO:211 Query: 321 WKVRVIGDDIAWMKFGADGRLYAINPEYGFVGAPGTSKTNPMAMASFQENTIFTNVAE 380  
SEQ ID NO:212 WKVR +GDDIAWMKFG DGRLYAINPE GFFGVAPGTS+KTNPMA+A+FG+N+IFTNVAE  
SEQ ID NO:213 Sbjct:19042 WKVRCVGGDDIAWMKFGEDGRLYAINPEAGFFGVAPGTSNKTNPMAVATFQKNSIFTNVAE 19221

SEQ ID NO:211 Query: 381 TADGEYFWEGLEHEVNKPKVDMINWLGEFPHIGDESKAAHPNS 423  
SEQ ID NO:212 TA+GEYFWEGLE E+ + VD+ WLGE WHIG+ AAHPNS  
SEQ ID NO:213 Sbjct: 19222 TANGEYFWEGLEDEIADKNVDITTWLGEKWHIGEPGVAHPNS 19350

Score = 657 (305.1 bits), Expect = 0.0, Sum P(5) = 0.0  
Identities = 120/173 (69%), Positives = 144/173 (83%), Frame = +1

SEQ ID NO:214 Query: 32 KGDFVSLPKHVQRFVAAEKAELMKPSAIFICDGSQNEADELIARCVERGVLPKAYKNY 91  
SEQ ID NO:215 +GDF LP VQRF+AEKAELM+P IFICDGSQ+EADELI + +ERG+L L+AY+NYY  
SEQ ID NO:216 Sbjct:18181 QGDFHLLPAKVQRFIAEKAELMRPGIFICDGSQHEADELIDKLIERGMLSKLEAYENNY 18360

SEQ ID NO:214 Query: 92 LCRTDPRDVARVESKTWMITPEKYDSVCHTPEGVKPMQWSPDEFKELDDRFPGCMA 151  
SEQ ID NO:215 +CRTDP+DVARVESKTWM+T KYD+V HT EGV+P+MG W++P++ ELD RFPGCMA  
SEQ ID NO:216 Sbjct:18361LCRTDPKDVARVESKTWMVTKNKYDTVHTKEGVPEIMGHWLAPEDLATELDSRFPGCMA 18540

SEQ ID NO:214 Query: 152 GRTMYVIPYSMGPVGGPLSKIGIELTSDYVVLICMRIMTRMGEPVLKALAKNN 204  
SEQ ID NO:215 GR MYVIP+SMGPVGGPLSKIGI+LTDS+YVVL MRIMTR+ V AL +  
SEQ ID NO:216 Sbjct: 18541 GRIMYVIPFSMGPVGGPLSKIGIQLTDSNYVVLICMRIMTRVNDVWDALGNQD 18699

Score = 453 (210.3 bits), Expect = 0.0, Sum P(5) = 0.0  
Identities = 77/107 (71%), Positives = 90/107 (84%), Frame = +1

SEQ ID NO:217 Query: 424 RFTAPAGQCPIIHPDWEKPEGVPIDAIIFGGRRPEGVPLVFESRSVWHGIFVGACVKSEA 483  
SEQ ID NO:218 RF APA QCPIIHPDWE P+GVPI+AIIFGGRRP+GVPL++E+ SW HG+F G+C+KSEA  
SEQ ID NO:219 Sbjct:19396 RFAAPANQCPIIHPDWESPQGVPIEAIIFGGRRPQGVPLIYETNSWEHGVTGSLCKSEA 19575

SEQ ID NO:217 Query: 484 TAAAEHTGKQVMHDPAMRPFMGYNFGRYMRHWMKLGQPPHKVPKIF 530  
SEQ ID NO:218 TAAAE TGK VMHDPAMRPFMGYNFG+Y++HW+ L KV F  
SEQ ID NO:219 Sbjct: 19576 TAAAEFTGKTVMHDPAMRPFMGYNFGKYLQHWLCLKTDSRKVIDFF 19716

Score = 404 (187.6 bits), Expect = 0.0, Sum P(5) = 0.0  
Identities = 68/116 (58%), Positives = 89/116 (76%), Frame = +1

SEQ ID NO:220 Query: 526 VPKIFHVNWFRQSADHKFLWPGYGDNIRVIDWILRRCSGDATIAEETPIGFIPKKGTINL585  
SEQ ID NO:221 +PKI+HVNWFR+ +++KFLWPG+GDNIRVIDWI+RR G+ I ETPIG +P KG+INL  
SEQ ID NO:222 Sbjct: 19750 MPKIYHVNWFRKDSNNKFLWPGFGDNIRVIDWIIRRLDGEQEIGVETPIGTVPKGSINL 19929

SEQ ID NO:220 Query: 586 EGLPNVNWDELMSIPKSYWLEDVMVETKTFENQVGSDDLPEIAKELEAQTERIKAL 641  
SEQ ID NO:221 EGL VNWDELMS+P YW +D E + F + QVG DLP + E++AQ +R++ L  
SEQ ID NO:222 Sbjct: 19930 EGLGEVNWDELMSVPADYWKQDAQEIRKFLDEQVGEDLPEPVRAEMDAQEKRVQTL 20097

Score = 69 (32.0 bits), Expect = 0.0, Sum P(5) = 0.0  
Identities = 15/36 (41%), Positives = 21/36 (58%), Frame = +1

SEQ ID NO:223 Query: 5 SLSHFKDDDDFAVVSEVVTHKQNHIPVIKGFVSLPK 40  
SEQ ID NO:224 SL +D F VV+EVV + H+P++K F S K  
SEQ ID NO:225 Sbjct: 14722 SLRQISEDFAFYVVNEVVMKRLGHVPILKVIFESSEK 14829

Score = 39 (18.1 bits), Expect = 6.9e-244, Sum P(4) = 6.9e-244  
Identities = 9/25 (36%), Positives = 11/25 (44%), Frame = +3

SEQ ID NO:226 Query: 148 GCMAGRTMYVIPYSMPVGGPLSKI 172  
SEQ ID NO:227 GC R + V P S PL K+  
SEQ ID NO:228 Sbjct: 8040 GCSGRRVLCVCPCSHSSSALPLQKV 8114

Score = 38 (17.6 bits), Expect = 4.0e-285, Sum P(5) = 4.0e-285  
Identities = 7/16 (43%), Positives = 9/16 (56%), Frame = +1

SEQ ID NO:229 Query: 588 LPNVNWDELMSIPKSY 603  
SEQ ID NO:230 L + NW +S P SY  
SEQ ID NO:231 Sbjct: 22654 LESFNWFSFVSCPDY 22701

Score = 37 (17.2 bits), Expect = 2.0e-48, Sum P(3) = 2.0e-48  
Identities = 6/14 (42%), Positives = 9/14 (64%), Frame = +1

SEQ ID NO:232 Query: 117 SVCHTPEGVKPMMG 130  
SEQ ID NO:233 +V H P ++P MG  
SEQ ID NO:234 Sbjct: 19603 TVMHDPAMRPFMG 19644

## Acetyl coa carboxylase

>W09B6

Length = 32,900

Plus Strand HSPs:

Score = 562 (259.1 bits), Expect = 0.0, Sum P(14) = 0.0  
Identities = 109/197 (55%), Positives = 138/197 (70%), Frame = +2

SEQ ID NO:235 Query: 1951 SGFFDYGSFSEIMQPWAQTVVVGRRARLGGIPVGVVAVETRTVELSVPADPANLDSEAKII 2010  
SEQ ID NO:236 z +G D SF EI WA+++V GRARL GIP+GVV+ E R VPADPA S+ +  
SEQ ID NO:237 Sbjct:28280 TGICDTMSFDEICGDWAKSIVAGRARLCGIPIGVVSSEFRNFSTIVPADPAIDGSQVQNT 28459

SEQ ID NO:235 Query: 2011 QQAGQVWFPSAFKTYQAIKDFNREGLPLMVFANWRGFSGGMKDMYDQVLKFGAYIVDGL 2070  
SEQ ID NO:236 Q+AGQVW+PDSAFKT +AI D N+E LPLM+ A+ RGFSGG KDMYD VLKFGA IVD L  
SEQ ID NO:237 Sbjct:28460 QRAGQVWYPDSAFKTAEAINDLNKENLPLMIASLRGFSGGKDMYDMLKFGAQIVDAL 28639

SEQ ID NO:235 Query: 2071 RECSQPMVYIPPAELRGGSWVIDPTINPRHMEMYADRESRGSVLEPEGTVEIKFRKK 2130  
SEQ ID NO:236 ++PV+VYIP ELRGG+W V+D I P + + AD +SRG +LEP V IKFRK  
SEQ ID NO:237 Sbjct: 28640 AVYNRPVIVYIPEAGELRGGAWAVLDSKIRPEFIHLVADEKSRGGILEPNAVVGIFKRKP 28819

SEQ ID NO:235 Query: 2131 DLVKTMRRVDPVYIRLA 2147  
SEQ ID NO:236 +++ M+R DP Y +L+  
SEQ ID NO:237 Sbjct: 28820 MMEMMKRSDPTYSKLS 28870

Score = 357 (164.6 bits), Expect = 0.0, Sum P(14) = 0.0  
Identities = 68/124 (54%), Positives = 89/124 (71%), Frame = +2

SEQ ID NO:238 Query:303 VGYPVMIKASEGGGGKGIRKVNADDPNLFRQVQAEVPGSPIFVMRLAKQSRHLEVQIL 362  
SEQ ID NO:239 +G+P+MIKASEGGGGKGIRK +DF ++F +V EV GSPIF+M+ +RH+EVQ+L  
SEQ ID NO:240 Sbjct:23264IGFPLMIKASEGGGGKGIRKCTKVEDFKSMFEEVAQEVQGSPIFLMKCVDGARHIEVQLL 23443

SEQ ID NO:238 Query: 363 ADQYGNALSLFGRDCSVQRRHQKXXXXXXXXXXXXXVFEHMEQCAVKLAKMVGYSAGTV 422  
SEQ ID NO:239 AD+Y N IS++ RDCS+QRR QK + + M++ AV+LAK VGY SAGTV  
SEQ ID NO:240 Sbjct:23444ADRYENVISVYTRDCSIQRRCKIIEEAPAIASSHIRKSMQEDAVRLAKYVGYESAGTV 23623

SEQ ID NO:238 Query: 423 EYLY 426  
SEQ ID NO:239 EYLY  
SEQ ID NO:240 Sbjct: 23624 EYLY 23635

Score = 345 (159.1 bits), Expect = 0.0, Sum P(14) = 0.0  
Identities = 65/116 (56%), Positives = 86/116 (74%), Frame = +2

SEQ ID NO:241 Query:1787 KEEGLGAENLRGSGMIAGESSLAYDEIITISLVTCTRAIGIGAYLVRLGQRTIQVENSILI 1846  
SEQ ID NO:242 K E +G ENL+GSG+IAGE++ AY E+ T VT R++GIGAY RL R +Q + SHLI  
SEQ ID NO:243 Sbjct:27794 KNEKIGVENLQSGSLIAGETARAYAEVPTYCYVTGRSVGIGAYTARLAHRIVQHKQSHLI 27973

SEQ ID NO:241 Query: 1847 LTGAGALNKVLGREVYTSNNQLGGIIMHNNGVTHCTVCDDFEGVFTVLHWLSYMP 1902  
SEQ ID NO:242 LTG ALN +LG++VYTSNNQLGG ++M NGVTH V +D EG+ V+ W+S++P  
SEQ ID NO:243 Sbjct: 27974 LTGYEALNTLLGKKVYTSNNQLGGPEVMFRNGVTHAVVDNDLEGIKAVIRWMSFLP 28141

Score = 319 (147.1 bits), Expect = 0.0, Sum P(14) = 0.0  
Identities = 59/119 (49%), Positives = 80/119 (67%), Frame = +2

SEQ ID NO:244 Query: 503 HVIAARITSENPDEGFKPSSGTQVQLNFRSNKNVWGYFSVAAAGGLHEFADSQFGHCFWS 562  
SEQ ID NO:245 H IAARIT ENPD+ F+PS+G V E+NF S+++ W YFSV +H+FADSQFGH F+

SEQ ID NO:246 Sbjct:23870 HAIAARITCENPDDSPSTGKVYEINFPSSQDAWAYFSVGRGSSVHQFADSQFGHIFTR 24049

SEQ ID NO:244 Query: 563 GENREEAISNMVVALKELSIRGDFRTTVEYLIKLLTESFQLNRIDTGWLDRLIAEKVQ 621

SEQ ID NO:245 G +R EA++ M LK ++IR F T V YL+ L+ F N +T WLD+ IA K++

SEQ ID NO:246 Sbjct: 24050 GTSRTEAMNTMCSTLKHMTIRSSFPTQVNYLVDLMDADFINNAFNTQWLDKRIAMKIK 24226

Score = 303 (139.7 bits), Expect = 0.0, Sum P(14) = 0.0

Identities = 55/90 (61%), Positives = 70/90 (77%), Frame = +2

SEQ ID NO: 247 Query: 178 PGGANNNNYANVELILDIAKRIPVQAVWAGWGHASENPKLPELLLKNGIAFMGPPSQAMW 237

SEQ ID NO: 248 P G N NN+ANV+ IL A + V AVWAGWGHASENP LP L + IAF+GPP+ AM+

SEQ ID NO: 249 Sbjct: 22886 PSGTNKNFANVDEILKHAIKYEVDVAVWAGWGHASENPDLPRRLNDHNIAFIGPPASAMF 23065

SEQ ID NO: 247 Query: 238 ALGDKIASIVAQTAGIPTLPWSGSGLRVD 267

SEQ ID NO: 248 +LGDKIAS+I+AQT G+PT+ WSGSG+ ++

SEQ ID NO: 249 Sbjct: 23066 SLGDKIASTIIAQTGVPTVAWSGSGITME 23155

## Trehelase

>C23H3

Length = 39,721

Minus Strand HSPs:

Score = 227 (104.5 bits), Expect = 1.0e-95, Sum P(6) = 1.0e-95  
Identities = 36/67 (53%), Positives = 51/67 (76%), Frame = -2

SEQ ID NO:250 Query: 2 VIKNLGYMVDNHGFVPNGGRVYYLTRSQPPLTPMVYEYVMSTGDLDFVMEILPTLDKEY 61  
SEQ ID NO:251 +I N +++++ GFVPNGGRVYYL RSQPP PMVYEEY++T D+ V +++P ++KEY  
SEQ ID NO:252 Sbjct:9798 MILNFAHIIETYGFPNGGRVYYLRRSQPPFFAPMVYEEYLATQDIQLVADLIPVIEKEY 9619

SEQ ID NO:250 Query: 62 EFWIKNR 68  
SEQ ID NO:251 FW + R  
SEQ ID NO:252 Sbjct: 9618 TFWSEERR 9598

Score = 182 (83.8 bits), Expect = 1.0e-95, Sum P(6) = 1.0e-95  
Identities = 32/92 (34%), Positives = 55/92 (59%), Frame = -2

SEQ ID NO:253 Query: 146 MDSIRTWSIIPADLNAFCANARILASLYEAGDFKKVKVFEQRYTWAKREMRELHWNENET 205  
SEQ ID NO:254 + +I T +I+P DLNAF+C N I+ Y++ G+ K + R+T + ++ +  
SEQ ID NO:255 Sbjct: 9372 ISTIETTNIIVPVDLNAFLCYNMNIMQLFYKLTGNPLKHLEWSSRFTNFREAF TKVFYVPA 9193

SEQ ID NO:253 Query: 206 DGIWYDYDIELKTHSNQYYVSNAPLYAKCYD 237  
SEQ ID NO:254 WYDY++ TH+ ++ SNAVPL+++CYD  
SEQ ID NO:255 Sbjct: 9192 RKGWYDYNLRTLTHNTDFFASNAVPLFSQCYD 9097

Score = 178 (81.9 bits), Expect = 1.0e-95, Sum P(6) = 1.0e-95  
Identities = 37/102 (36%), Positives = 55/102 (53%), Frame = -2

SEQ ID NO:256 Query: 246 VHDYLERQGLLKYT KGLPTSLAMSSTQQWDKENAWPPMIHVMVIEGFRRTGDIKLMKVAEK 305  
SEQ ID NO:257 V++ ++ G G+PTS+ + QQWD N W PM HM+IEG R + + L + A  
SEQ ID NO:258 Sbjct: 9069 VYNEMQNSGAFSIPGGIPTSMNEETNQQWDFPNGWSPMNHMIIEGLRKSNNPILQQKFT 8890

SEQ ID NO:256 Query: 306 MATSWLTGTYQS FIRTHAMFEKYNVTPHTEETSGGGGGGEYEV 347  
SEQ ID NO:257 +A WL Q+F + M+EKYNV + + GG E +V  
SEQ ID NO:258 Sbjct: 8889 LAEKWLETNMQTFNVSDMWEKYNVKEPLGKLATGGEYEVQV 8764

Score = 169 (77.8 bits), Expect = 1.0e-95, Sum P(6) = 1.0e-95  
Identities = 29/58 (50%), Positives = 41/58 (70%), Frame = -2

SEQ ID NO:259 Query: 84 YQYKAKLKVPRPESYREDSELAEHLQTEAEKIQMWSEIASAAETGWDFSTRWFSQNGD 141  
SEQ ID NO:260 +QY+ + + PRPES+RED AEH T+ K Q + ++ SAAE+GWDFS+RWF + D  
SEQ ID NO:261 Sbjct: 9546 FQYRTEAETPRPESFREDVLSAEHFTTKDRKKQFFKDLGSAAESGWDFSSRWFKNHKD 9373

Score = 76 (35.0 bits), Expect = 1.0e-95, Sum P(6) = 1.0e-95  
Identities = 13/21 (61%), Positives = 15/21 (71%), Frame = -1

SEQ ID NO:262 Query: 348 QTGFGWTNGVILDLLDKYGDQ 368  
SEQ ID NO:263 Q GFGWTNG LDL+ Y D+  
SEQ ID NO:264 Sbjct: 8722 QAGFGWTNGAALDLIFTYSR 8660

Score = 45 (20.7 bits), Expect = 1.0e-95, Sum P(6) = 1.0e-95  
Identities = 10/24 (41%), Positives = 15/24 (62%), Frame = -1



SEQ ID NO:265 Query: 371 SSSTASKFSFSLSNITFVVFILYI 394  
SEQ ID NO:266 +SS++S F +S VF+LYI  
SEQ ID NO:267 Sbjct: 8545 TSSSSSTFGYSNLTITVFLYI 8474

Score = 38 (17.5 bits), Expect = 2.6e-98, Sum P(7) = 2.6e-98  
Identities = 7/7 (100%), Positives = 7/7 (100%), Frame = -2

SEQ ID NO:268 Query: 342 GGEYEVQ 348  
SEQ ID NO:269 GGEYEVQ  
SEQ ID NO:270 Sbjct: 8787 GGEYEVQ 8767

Score = 37 (17.0 bits), Expect = 1.6e-19, Sum P(4) = 1.6e-19  
Identities = 8/18 (44%), Positives = 10/18 (55%), Frame = -2

SEQ ID NO:271 Query: 217 KTHSNQYYVSNAVPLYAK 234  
SEQ ID NO:272 K ++ YYVS P Y K  
SEQ ID NO:273 Sbjct: 30345 KFTAHPYYVSRTPPRYHK 30292

>W05E10

Length = 31,273

Minus Strand HSPs:

Score = 224 (103.1 bits), Expect = 7.0e-90, Sum P(7) = 7.0e-90  
Identities = 43/67 (64%), Positives = 49/67 (73%), Frame = -1

SEQ ID NO:274 Query: 2 VIKNLGYMVDNHFVPNGGRVYYLTRSQPPLTPMVYEYVMSTGDLDFVMEILPTLDKEY 61  
SEQ ID NO:275 +I+NL MVD +GFVPNGGRVYYL RSQPP L MVYE Y+ T D FV E+LPTL KE  
SEQ ID NO:276 Sbjct:28957MIRNLASMVDKYGFPNGGRVYYLQRSQPPFLAAMVYELYEATNDKAFVAELLPTLLKEL28778

SEQ ID NO:274 Query: 62 EFWIKNR 68  
SEQ ID NO:275 FW + R  
SEQ ID NO:276 Sbjct: 28777 NFWNEKR 28757

Score = 192 (88.4 bits), Expect = 7.0e-90, Sum P(7) = 7.0e-90  
Identities = 31/84 (36%), Positives = 52/84 (61%), Frame = -3

SEQ ID NO:277 Query: 154 IIPADLNAFMCANARILASLYEAGDFKKVKVFEQRYTWAKREMRELHWNEDGIWYDYD 213  
SEQ ID NO:278 ++P DLN + C N I + LYE GD K ++F + + ++ + +N TDG WYDY+  
SEQ ID NO:279 Sbjct:2842 7VLPVDLNGLLCNMDIMEYLYEQIGDTKNSQIFRNKRADFRDTVQNVFYNRTDGTWYDYN 28248

SEQ ID NO:277 Query: 214 IELKTHSNQYYVSNAVPLYAKCYD 237  
SEQ ID NO:278 + ++H+ ++Y S AVPL+ CY+  
SEQ ID NO:279 Sbjct: 28247 LRTQSHNPRFYTSTAVPLFTNCYN 28176

Score = 125 (57.5 bits), Expect = 7.0e-90, Sum P(7) = 7.0e-90  
Identities = 20/48 (41%), Positives = 30/48 (62%), Frame = -2

SEQ ID NO 280 Query: 249 YLERQGLLKYTEKGLPTSLAMSSTQQWDKENAWPPMIHVMIEGFRTTGD 296  
SEQ ID NO 281 + ++ G+ Y G+PTS++ S QQWD N W P HM+IEG R + +  
SEQ ID NO 282 Sbjct: 28092 FFQKMGVFTYYPGGIPTSMSQESDQQWDFPNGWSPNNHMIIEGLRKSAN 27949

Score = 90 (41.4 bits), Expect = 7.0e-90, Sum P(7) = 7.0e-90  
Identities = 15/18 (83%), Positives = 18/18 (100%), Frame = -2

SEQ ID NO 283 Query: 120 EIASAAETGWDFSTRWFS 137  
SEQ ID NO 284 ++ASAAE+GWDFSTRWFS  
SEQ ID NO 285 Sbjct: 28566 DLASAAESGWDFSTRWFS 28513

Score = 89 (41.0 bits), Expect = 7.0e-90, Sum P(7) = 7.0e-90  
Identities = 18/40 (45%), Positives = 24/40 (60%), Frame = -1

SEQ ID NO 286 Query: 79 KQFPYYQYKAKLKVPRPESYREDSELAEHLQTEAEKIQMW 118  
SEQ ID NO 287 K F YQYK VPRPESYR D++ + L A++ Q +  
SEQ ID NO 288 Sbjct: 28732 KSFKVYQYKTASNVP RPESYRVDTONSAKLANGADQQQFY 28613

Score = 77 (35.4 bits), Expect = 7.0e-90, Sum P(7) = 7.0e-90  
Identities = 14/21 (66%), Positives = 16/21 (76%), Frame = -3

SEQ ID NO 289 Query: 348 QTGFGWTNGVILDLLDKYGDQ 368  
SEQ ID NO 290 Q GFGW+NG ILDLL Y D+  
SEQ ID NO 291 Sbjct: 24395 QDGFWSNGAILDLLLTYNDR 24333

Score = 51 (23.5 bits), Expect = 7.0e-90, Sum P(7) = 7.0e-90  
Identities = 11/27 (40%), Positives = 16/27 (59%), Frame = -3

SEQ ID NO 292 Query: 365 YGDQFASSTASKFSFSLSNITFVVF 391  
SEQ ID NO 293 Y FASSS AS FS +++ F + +  
SEQ ID NO 294 Sbjct: 2846 YN\*PFASSSDASSCPSTNSVIFSILV 2766

Score = 41 (18.9 bits), Expect = 3.3e-93, Sum P(8) = 3.3e-93  
Identities = 7/9 (77%), Positives = 8/9 (88%), Frame = -2

SEQ ID NO 295 Query: 340 GGGGEYEVQ 348  
SEQ ID NO 296 G GGEY+VQ  
SEQ ID NO 297 Sbjct: 24468 GSGGEYDVQ 24442

Score = 39 (18.0 bits), Expect = 2.0e-37, Sum P(5) = 2.0e-37  
Identities = 7/14 (50%), Positives = 8/14 (57%), Frame = -2

SEQ ID NO 298 Query: 221 NQYYVSNAVPLYAK 234  
SEQ ID NO 299 N YY+ V LY K  
SEQ ID NO 300 Sbjct: 4524 NHYYIIQMVS LYTK 4483

Score = 38 (17.5 bits), Expect = 4.0e-88, Sum P(7) = 4.0e-88  
Identities = 11/30 (36%), Positives = 13/30 (43%), Frame = -1

SEQ ID NO 301 Query: 367 DQFASSTASKFSFSLSNITFVVFILYIFS 396  
SEQ ID NO 302 DQF S SKFS + F +FS  
SEQ ID NO 303 Sbjct: 7588 DQFVISFICKFSSKNKKLYFCPSHFSLFS 7499

### In the Figures:

In response to the Draftperson's Patent Drawing Review, replace the originally filed informal drawings with the formal drawings submitted herewith.

In the Claims:

Amend claims 1 and 2 to read as follows.

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1. (Twice Amended) A method for identifying a compound that modulates DAF-18 expression or activity, comprising:

(a) providing a nematode, isolated nematode cell, or isolated mammalian cell expressing a nematode *daf-18* gene; and

E<sub>5</sub> (b) contacting said nematode, isolated nematode cell, or isolated mammalian cell with a candidate compound to determine the effect of said candidate compound on *daf-18* expression or activity, an alteration in said *daf-18* expression or activity following contact of said nematode, isolated nematode cell, or isolated mammalian cell with said candidate compound identifying said candidate compound as a modulatory compound.

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2. (Twice Amended) A method for identifying a compound that modulates PTEN expression or activity, comprising:

E<sub>4</sub> (a) providing a nematode or isolated nematode cell comprising a mutation in its endogenous *daf-18* gene;

(b) expressing in said nematode or isolated nematode cell a mammalian PTEN gene; and

(c) contacting said nematode or isolated nematode cell with a candidate compound to determine the effect of said candidate compound on PTEN expression or activity, an alteration in said PTEN expression or activity following contact with said candidate compound identifying said candidate compound as a modulatory compound.

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## REMARKS

Claims 1-5, 8, 10-17, 23, and 25-28 are under examination in the present case. Each of these claims is rejected under 35 U.S.C. § 112, first paragraph. Applicants note that the Office incorrectly identified claims 18-22 as pending. Claims 1 and 2 are further rejected under 35 U.S.C. § 112, second paragraph. The rejections are addressed below.

### Support for the amendments

Support for the amendments is found throughout the specification. No new matter has been added. For example, support for the amendment of claims 1 and 2 is found at claims 1 and 2 as originally filed, and at page 15, lines 20-25, and page 16, lines 1-14.

### Rejections under 35 U.S.C. § 112, first paragraph

#### *Written Description*

Claims 1-5, 12-15, and 23 stand rejected under 35 U.S.C. § 112, first paragraph, based on the assertion that the specification fails to provide a written description that conveys to the skilled artisan that Applicants were in possession of the claimed invention at the time of filing.

Claims 1-5 and 12-15 provide methods for compound identification that require expression of the *C. elegans* DAF-18 gene or PTEN. Claim 23 provides a transgenic nematode whose cells contain a transgene encoding a mammalian PTEN polypeptide. The Office bases the rejection of these claims on three grounds: (i) that Applicants have not disclosed *daf-18*/PTEN homologs from any and all nematodes, and any and all mammals; (ii) that Applicants have not disclosed PTEN mutational sites that exist in nature; and (iii) that Applicants have not described how the structure of nematode and human PTEN relates to the structure of PTEN in other nematode or mammalian species. This rejection is respectfully traversed.

*Disclosure of Any and All daf-18/PTEN Homologs is Not Required to Satisfy the Written Description Requirement.*

The Office asserts that Applicants' disclosure of *C. elegans daf-18* and human PTEN nucleic acid and amino acid sequences is insufficient to show that Applicants were in possession of the claimed genus, and instead indicates that a disclosure of any and all *daf-18*/PTEN homologs is required. Applicants disagree, and direct the Examiner's attention to M.P.E.P. 2163 II A 3ii, where it states,

The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by ...*disclosure of relevant identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics*, sufficient to show the applicant was in possession of the claimed genus...

Applicants have clearly satisfied these requirements.

*Characteristics of daf-18/PTEN*

In satisfaction of the written description requirement, Applicants have disclosed, in their specification, identifying characteristics of *daf-18*/PTEN, including functionally important structural features. For example, at Figures 40A and 40B, Applicants provide the amino acid and nucleic acid sequences of *C. elegans* DAF-18. At Figure 39A, Applicants provide a schematic diagram that illustrates the exon/intron structure of *daf-18* and highlights the exons encoding the functionally important phosphatase domain. At Figure 39B, Applicants show an alignment of *C. elegans daf-18* and human PTEN that indicates the probable phosphatase active sites. At page 109, lines 9-16, Applicants disclose that the amino acid sequence identity of DAF-18 and human PTEN is 38% within the phosphatase domain, and 90% (18/20) identical surrounding the Cys-(X)<sub>5</sub>-Arg putative phosphatase active site.

### *Mutational Sites*

In addition, and contrary to the Office's assertion, Applicants have also described, in their specification, mutations present in both *C. elegans daf-18* and human PTEN.

With respect to *daf-18*, for example, at page 109, lines 20-24, Applicants state:

A 30 base pair insertion mutation was detected in *daf-18(e1375)* (Figure 39A). This insertion mutation occurs within exon 4 and is predicted to insert 6 amino acids to the coding sequence before introducing a stop codon. The insertion is composed of a thirteen base pair repeat and two smaller repeat segments.

This insertion is predicted to truncate the carboxy terminal half of the protein (page 109, line 24, to page 110, line 3). Applicants conclude that *daf-18(e1375)* is unlikely to represent a null allele, since the phenotype of the *daf-18(1375)* mutant nematodes is less severe than nematodes in which *daf-18* has been inactivated by RNA-interference.

With respect to human PTEN, Applicants disclose that mutations within the PTEN phosphatase domain and carboxy terminal domain are oncogenic, and that such mutations are described in the Human Gene Mutation Database, and in Krawczak and Cooper, *Trends in Genetics* 13:121-12, 1997. In addition, Applicants disclose, at page 110, lines 8-11, that mutations within the carboxy terminal of PTEN and DAF-18 likely effect protein localization, since these regions are not conserved between *C. elegans* and mammals.

### *daf-18/PTEN homologs*

Finally, Applicants have demonstrated that the structural similarities between the members of the *daf-18/PTEN* family are echoed in functional relatedness. As stated in Dr. Ruvkun's Declaration, although *C. elegans* and humans are evolutionarily distant organisms, *C. elegans daf-18*, human PTEN and proteins are highly related. In fact, Applicants have found that the two proteins are so closely related that human PTEN is able to functionally substitute for *C. elegans* DAF-18 *in vivo*. Given this result, it is fully

expected that other highly related nematode or mammalian DAF-18/PTEN proteins would also substitute for *C. elegans* DAF-18.

Human PTEN is representative of mammalian PTEN's generally, since many other mammalian PTEN proteins are as closely related to *C. elegans* DAF-18 as human PTEN protein is. This is demonstrated when *C. elegans* DAF-18 is used in a BLAST search of mammalian genomes. DAF-18 identifies PTEN homologs in many mammalian species, including human (Accession No.: NP\_000305.1) ( $6e^{-39}$ ), rat (Accession No.: NP\_113794.1) ( $5e^{-39}$ ), mouse (Accession No.: NP\_032986.1) ( $6e^{-39}$ ), and cow (Accession No.: AAG33701.1) ( $1e^{-20}$ ) (Ruvkin Declaration, paragraph 4). The probability of a random alignment between *C. elegans* DAF-18 and any of these mammalian PTEN proteins is extremely low.

Moreover, *C. elegans* DAF-18 and human PTEN share a conserved phosphatase domain, which is the hallmark of a DAF-18/PTEN protein. Given Applicants' finding that human PTEN substitutes for DAF-18 *in vivo*, it is clear that *C. elegans* DAF-18 and human PTEN also share a common enzymatic activity, i.e., both degrade the second messenger phosphatidyl inositol triphosphate (PIP3). Other DAF-18/PTEN proteins are expected to share this enzymatic activity given that their phosphatase domains are as closely related to *C. elegans* DAF-18 as is human PTEN. Indeed, as evidenced by the exemplary alignments below between the domain from *C. elegans* and the human, rat, and mouse domains, it may be concluded that the DAF-18/PTEN phosphatase domain is highly conserved among mammalian DAF-18/PTEN proteins (Ruvkin Declaration, paragraphs 3-5).

### C. elegans DAF-18 Alignment with Human PTEN

gi|2811005|sp|O00633|PTEN HUMAN Protein-tyrosine phosphatase PTEN (Mutated in multiple advanced cancers 1)

Length = 403

Score = 161 bits (537), Expect = 6e-39

Identities = 90/207 (43%), Positives = 126/207 (60%), Gaps = 1/207 (0%)

Query: 48 IFRTAVSSNRCRTEYQNIDLDCAIYITDRIIAIGYPATGIEANFRNSKVQTQQFLTRRHGK 107  
I + VS N+ R + DLD YI IIA+G+PA +E +RN+ +FL +H K  
Sbjct: 4 IIKEIVSRNKRRYQEDGFDLDLTYYIPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKH-K 62

Query: 108 GNVKVFNLRGYYDYDADNFDGNVICFDMTDHPPSLELMAPFCREAKEWLEADDKHVIAV 167  
+ K++NL +YD F+ V + DH+PP LEL+ PFC + +WL DD HV A+  
Sbjct: 63 NHYKIYNLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPFCELDQWLSEDDNHVAAI 122

Query: 168 HCKAGKGRTGVMICALLIYINFYSPRQILDYYSIIRTKNNKGV TIPSQRRYIYYYHKLR 227  
HCKAGKGRTGVMICA L++ + ++ LD+Y +RT++ KGV TIPSQRRY+YYY L  
Sbjct: 123 HCKAGKGRTGVMICAYLLHRGKFLKAQEALDFYGEVTRDKKGV TIPSQRRYVYYYSYLL 182

Query: 228 ERELNYLPLRMQLIGVYVERPPKTWGG 254  
+ L+Y P+ + + E P GG  
Sbjct: 183 KNHLDYRPVALLFHKMMFETIPMFSGG 209

### C. elegans DAF-18 Alignment with Rat PTEN

gi|13928830|ref|NP\_113794.1| phosphatase and tensin homolog; phosphatase and tensin homolog (mutated in multiple advanced cancers 1) [Rattus norvegicus]

Length = 403

Score = 161 bits (537), Expect = 5e-39

Identities = 90/207 (43%), Positives = 126/207 (60%), Gaps = 1/207 (0%)

Query: 48 IFRTAVSSNRCRTEYQNIDLDCAIYITDRIIAIGYPATGIEANFRNSKVQTQQFLTRRHGK 107  
I + VS N+ R + DLD YI IIA+G+PA +E +RN+ +FL +H K  
Sbjct: 4 IIKEIVSRNKRRYQEDGFDLDLTYYIPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKH-K 62

Query: 108 GNVKVFNLRGYYDYDADNFDGNVICFDMTDHPPSLELMAPFCREAKEWLEADDKHVIAV 167  
+ K++NL +YD F+ V + DH+PP LEL+ PFC + +WL DD HV A+  
Sbjct: 63 NHYKIYNLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPFCELDQWLSEDDNHVAAI 122

Query: 168 HCKAGKGRTGVMICALLIYINFYSPRQILDYYSIIRTKNNKGV TIPSQRRYIYYYHKLR 227  
HCKAGKGRTGVMICA L++ + ++ LD+Y +RT++ KGV TIPSQRRY+YYY L  
Sbjct: 123 HCKAGKGRTGVMICAYLLHRGKFLKAQEALDFYGEVTRDKKGV TIPSQRRYVYYYSYLL 182

Query: 228 ERELNYLPLRMQLIGVYVERPPKTWGG 254  
+ L+Y P+ + + E P GG  
Sbjct: 183 KNHLDYRPVALLFHKMMFETIPMFSGG 209



### C. elegans DAF-18 Alignment with Mouse PTEN

gi|2811066|sp|O08586|PTEN\_MOUSE PROTEIN-TYROSINE PHOSPHATASE PTEN (MUTATED  
IN MULTIPLE ADVANCED CANCERS 1)

Score = 161 bits (537), Expect = 6e-39

Identities = 90/207 (43%), Positives = 126/207 (60%), Gaps = 1/207 (0%)

Length = 403

Query: 48 IFRTAVSSNRCRTEYQNIDLDCAYITDRIIAIGYPATGIEANFRNSKVQTQQFLTRRHGK 107

I + VS N+ R + DLD YI IIA+G+PA +E +RN+ +FL +H K

Sbjct: 4 IIKEIVSRNKRRYQEDGFDLDTYIYPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKH-K 62

Query: 108 GNVKVFNLRGYYDADNFDGNVICFDMTDHHPPSLELMAPFCREAKEWLEADDKHVIAV 167

+ K++NL +YD F+ V + DH+PP LEL+ PFC + +WL DD HV A+

Sbjct: 63 NHYKIYNLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPFCELDQWLSEDDNHVAAI 122

Query: 168 HCKAGKGRGTGVMICALLIYINFYSPRQILDYYSIIRTKNNKGV TIPSQRRYIYYHKL R 227

HCKAGKGRGTGVMICA L++ + ++ LD+Y +RT++ KGV TIPSQRRY+YYY L

Sbjct: 123 HCKAGKGRGTGVMICAYLLHRGKFLKAQEALDFYGEVTRD KKGVTIPSQRRYVYYYSYLL 182

Query: 228 ERELNYLPLRMQLIGVYVERPPK TWGG 254

+ L+Y P+ + + E P GG

Sbjct: 183 KNHLDYRPVALLFHKMMFETIPMFSGG 209

In fact, rat, mouse, and human PTENs share 43% amino acid identity (Ruvkun Declaration, paragraph 5) with *C. elegans* DAF-18 in the phosphatase region. Given the conserved nature of the phosphatase domain among all DAF-18/PTEN proteins, it would be expected that most if not all nematode and mammalian DAF-18/PTEN proteins degrade PIP3 and are functionally similar to *C. elegans* DAF-18.

Furthermore, those skilled in the art accept that the conserved phosphatase domain is a hallmark that is conserved among all DAF-18/PTEN proteins. This is evidenced by Exhibit A (Lee et al., Crystal Structure of the PTEN Tumor Suppressor: Implications for Its Phosphoinositide Phosphatase Activity and Membrane Association, *Cell* 99:323-334, 1999). In this publication, Lee discloses the crystal structure of PTEN. With respect to the phosphatase domain, at page 323, right column, second paragraph, Lee states, "The 403-amino acid PTEN protein contains the signature motif HCXXGXXR present in the active sites of protein tyrosine phosphatases (PTPs) and dual specificity protein phosphatases." This "signature motif" is highly conserved between *C. elegans* and

mammalian PTEN proteins and has been underlined in the alignments above (Ruvkun Declaration, paragraph 6).

Those skilled in the art further accept that mammalian PTEN and *C. elegans* DAF-18 fulfill equivalent functions in conserved pathways, also as evidenced by Lee (at page 323, right column third paragraph):

A role for the PI(3, 4, 5)P<sub>3</sub> phosphatase activity of PTEN in its tumor suppressor function has been supported by several observations. Tumor cell lines with mutant PTEN have elevated levels of PI(3,4,5)P<sub>3</sub> and of Akt activity (Stambolic et al., 1998), and the introduction of wild-type PTEN reduces levels of both (Li and Sun, 1998). In *C. elegans*, a pathway that contains the PI<sub>3</sub> kinase and Akt homologs also contains the DAF-18 gene that has homology to PTEN (Ogg and Ruvkun, 1998). Finally, the Gly129Glu tumor-derived mutation that maps to the phosphatase signature motif eliminates PTEN's lipid phosphatase activity but not its protein phosphatase activity...

Clearly, based on Applicants' disclosure, Lee et al., as a representative of those skilled in the art, accept that *C. elegans* DAF-18 and mammalian PTEN are homologs that function in conserved pathways (Ruvkun Declaration, paragraph 7).

In sum, Applicants have shown that *C. elegans daf-18* and human PTEN are representative of DAF-18/PTEN proteins generally. In addition, Applicants have provided a detailed description of DAF-18/PTEN, including the nucleic acid and amino acid sequences of *daf-18*, an alignment of DAF-18 and PTEN, identification of the structurally and functionally important phosphatase domain that is characteristic of all DAF-18/PTEN proteins, and identification of mutations in *C. elegans daf-18* and human PTEN. Moreover, Applicants have shown that human PTEN and *C. elegans daf-18* as species are clearly representative of the genus. This is all that is required by the case law; the written description rejection should be withdrawn.

### *Scope of Claims 23 and 25-28*

Claims 23 and 25-28 are rejected based on the assertion that the specification fails to enable the skilled artisan to make and use the invention commensurate in scope with the claims. More specifically, the Office asserts that the phenotype of a transgenic nematode expressing a DAF-18/PTEN homolog from any nematode other than *C. elegans* or any mammal other than a human is unpredictable given (i) that the phenotype of a transgenic animal is determined by complex interactions between genetics and the environment; (ii) that the phenotype of a transgenic animal is influenced by the animal's genetic background; (iii) that transgene expression is subject to positional effects; (iv) that biochemical pathways are plastic and able to compensate for alterations in pathway components; and (v) that transgene expression is influenced by the expression vector that is used. This rejection is respectfully traversed.

### *Interactions between Genetics and Environment*

The Office cites Wood et al., (*Comparative Medicine*, 50:12-15, 2000, "Wood") to support its assertion that the phenotype of a transgenic animal is determined by a complex interaction between genetics and environment.

Wood teaches that specific paradigms are useful in assessing the phenotype of a genetically modified rodent (page 12, left column, second paragraph).

The purpose of this paper is to describe a general scheme of approaching the overall phenotype assessment of the large number of genetically altered or spontaneous *mutant mice*, as well as other *rodent models* currently being developed.

The claimed invention is directed to a transgenic nematode. This claimed invention does not require the production of a genetically modified rodent and the issues raised in Wood are therefore of little or no relevance.

### *Genetic Background*

The Office cites Sigmund (*Arterioscler. Thromb. Vasc. Biol.*, June 2000:1425-1429, 2000, "Sigmund") to support its assertion that genetic background affects the phenotype of a transgenic nematode. Sigmund teaches that the effects of genetic variability in mutant mouse models can be minimized by using strategies to minimize genetic variation between experimental and control mice. These strategies include the use of isogenic strains or congenic strains of mice, and successive back-crossing of genetically altered mice (page 1426, right column, second paragraph, to page 1428, left column, first paragraph), as well as the evaluation of large numbers of mice to reduce variability associated with epigenetic effects.

Once again, Applicants note that the claimed invention is directed to nematodes, not mice. Moreover, the methods taught by Sigmund for minimizing genetic variability between experimental and control mice, i.e., the use of isogenic strains are routine in nematode biology as noted in Dr. Ruvkun's Declaration, at paragraph 8.

### *Genetic Interaction among DAF Genes*

The Office asserts that genetic interactions among *daf* genes and their gene products is well-studied only in *C. elegans*, and cites Larsen et al. (*Genetics*, 139:1567-1583, 1995, "Larsen") in support of this position. Larsen teaches that *daf* genes regulate development and longevity in *C. elegans*. Larsen does not address whether the *daf* pathway is understood in organisms other than *C. elegans*. In fact, with respect to *daf* genes in other organisms, Larsen states:

*daf* genes encode signal transduction components that are conserved between species to the degree that a human ligand (bone morphogenetic protein, BMP-4) can interact effectively with the nematode *daf-4* receptor...Hence, it seems possible that elucidation of efficient life maintenance mechanisms controlled by *daf* genes could reveal insights into mechanism affecting human life span (Larsen et al., *supra*, page 1582, left column, lines 1-8).

In fact, Larsen supports Applicants' position that components of the *daf* pathway (e.g., *daf-18*) are highly conserved between nematodes and humans, and that interactions among *daf* gene products are also highly conserved.

Moreover, as applied to *daf-18* and PTEN, specifically, this general concern is unwarranted. Applicants have demonstrated that these proteins are functionally interchangeable. Thus, there can be no question that interactions between the *C. elegans* *daf* pathway and mammalian homologs occur.

This position is further supported by Exhibit A (Lee et al., Crystal Structure of the PTEN Tumor Suppressor: Implications for Its Phosphoinositide Phosphatase Activity and Membrane Association, *Cell* 99:323-334, 1999). In this publication by a third party, Lee accepts this conservation between the *C. elegans* and human pathways, stating:

A role for the PI(3, 4, 5)P<sub>3</sub> phosphatase activity of PTEN in its tumor suppressor function has been supported by several observations. Tumor cell lines with mutant PTEN have elevated levels of PI(3,4,5)P<sub>3</sub> and of Akt activity (Stambolic et al., 1998), and the introduction of wild-type PTEN reduces levels of both (Li and Sun, 1998). In *C. elegans*, a pathway that contains the PI<sub>3</sub> kinase and Akt homologs also contains the DAF-18 gene that has homology to PTEN (Ogg and Ruvkun, 1998). Finally, the Gly129Glu tumor-derived mutation that maps to the phosphatase signature motif eliminates PTEN's lipid phosphatase activity but not its protein phosphatase activity...(at page 323, right column, third paragraph).

Clearly, Lee et al. accepts that *C. elegans* DAF-18 and mammalian PTEN function in conserved pathways.

#### *Integration Site*

The Office asserts that transgene integration site and sequences present in a transgenic construct can effect transgene expression, and cites Wall (*Theriogen.*, 45:57-68, 1996, "Wall") in support of this position. Wall discusses technical difficulties associated with transgenic livestock production. Once again, Applicants point out that the invention as claimed is directed to transgenic nematodes, and as discussed in more

detail below, Dr. Ruvkun's Declaration states that transgene integration is not necessary to the production of a transgenic nematode.

#### *Cis-Acting Elements*

The Office cites Pursel et al. (*J. Reproductive Fertility, Supplement*, 40: 235-245, 1990) to support their position that cis acting elements have an unpredictable effect on transgene expression. Pursel teaches that eleven different murine, bovine, and ovine regulatory sequences were used to express growth hormone in transgenic pigs, and that the expression levels of growth hormone varied depending on the regulatory sequence used. Once again, Applicants point out that the claimed invention features transgenic nematodes and Pursel's teaching regarding transgenic pigs is not of relevance, given that suitable nematode expression constructs are known to the skilled artisan, and Applicants have provided a working example showing that a nematode expression construct containing nematode daf-18 regulatory sequences directed expression of a human gene at levels and in tissues that were sufficient to rescue the mutant nematode phenotype.

#### *Expression Vector*

The Office cites Kappel et al. (*Current Opinion in Biotechnology* 3:548-553, 1992) to support its position that the phenotype of a transgenic animal depends on the expression vector used to produce the transgenic animal. Kappel reviews the use of transgenic mice and knock-out mice to study mammalian genes of unknown function. Once again, Applicants note that the claimed invention features transgenic nematodes and Kappel's teaching regarding transgenic mice is not of relevance.

#### *Transgenic Nematode Production*

In truth, the production of transgenic nematodes expressing mammalia PTEN genes is fully enabled by Applicants' specification. As stated in Dr. Ruvkun's Declaration, transgenic nematodes are inherently different from transgenic mammals.

Producing a transgenic nematode is a routine matter, requiring no more than standard methods. Transgenic *C. elegans* are made by microinjecting plasmid or linear DNA with a selectable marker. *In vivo*, the DNA is assembled into concatamers that are properly regulated. In contrast to mammalian transgenes, it is not necessary for a transgene to be integrated into the *C. elegans* genome. Thus, *C. elegans* extra-genomic transgenes are not subject to the problems associated with mammalian transgene integration.

Transgenic nematodes are used routinely to identify a coding region that corresponds to a genetically-defined locus, and to rescue mutations by complementation. In nematodes, transgene expression is reliably used to produce a wild-type phenotype. Moreover, all experiments are routinely carried out in isogenic strains (Ruvkun Declaration, paragraph 8).

Accordingly, a transgenic nematode expressing virtually any mammalian or nematode DAF-18/PTEN protein could easily be produced using no more than routine methods. As described in Dr. Ruvkun's previous Declaration, mailed on July 10, 2000, human PTEN cDNA was placed under the control of approximately 1.0 kb of *daf-18* 5' flanking sequence and approximately 2.4 kb of *daf-18* 3' flanking sequence and expressed in transgenic *C. elegans* (*daf-2;daf-18* double mutants). This method was fully enabled at the time of filing, and a working example describing transgenic worm production is found in Applicants' specification, at page 40, lines 22-25, and page 41, lines 1-14. To produce a transgenic nematode expressing virtually any DAF-18/PTEN protein, the skilled artisan could merely swap the desired DAF-18/PTEN-expressing nucleic acid for the human PTEN cDNA present in the *C. elegans* expression vector. Given these results showing that human PTEN expression can substitute for DAF-18 in *daf-18* deficient nematodes, and given the level of conservation present in mammalian PTEN proteins, particularly within the phosphatase domain, the skilled artisan would fully expect the phenotype of a DAF-18/PTEN-expressing transgenic nematode to resemble a human PTEN-expressing transgenic nematode (Ruvkun Declaration, Paragraph 9).

The scope of the present claims is therefore appropriate, and the enablement rejection as applied to claims 23 and 25-28 should be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1 and 2 are rejected under 35 U.S.C. § 112, second paragraph, based on the assertion that they omit essential steps.

Claim 1 has been amended, in accordance with the Office's suggestion, to recite "a candidate compound to determine the effect of said compound on Daf-18 expression or activity." Claim 2 has also been amended, in accordance with the Office's suggestion, to recite "a candidate compound to determine the effect of said compound on PTEN expression or activity." These rejections may now be withdrawn.

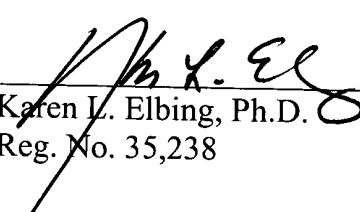


CONCLUSION

If there are any charges or any credits, please apply them to Deposit Account No.  
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Respectfully submitted,

Date: 20 November 2002

  
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